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Habitat Utilization and Salinity Tolerance of the Sandbar Shark, Carcharhinus plumbeus, in Virginia

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Leonard Jason Reed Pace
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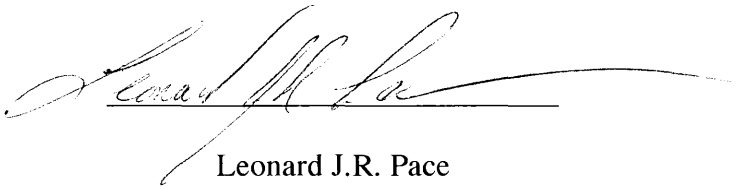

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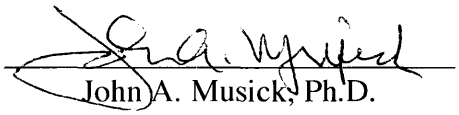

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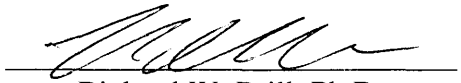

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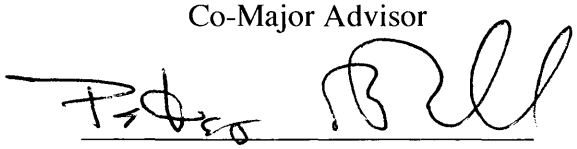

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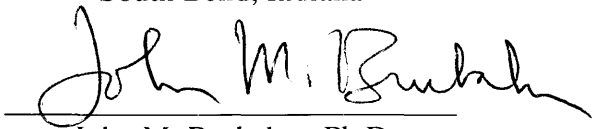

John M. Brubaker, Ph.D

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I thank all those who played their part... In helping me to play with sharks... I really had fun... And struggled a ton... And am proud to have made my mark.

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EXPLANATION OF THESIS CONSTRUCTION

This thesis has been constructed in a format conducive with submission to the Journal of Experimental Marine Biology and Ecology. Formatting of the body of this work was designed intentionally to be in manuscript form. Appendices have been included to further explain methodology and to enhance the presentation of knowledge gained while completing the degree program.

Tolerances of sandbar (*Carcharhinus plumbeus*) and dusky (*Carcharhinus obscurus*) sharks to reduced salinities

Abstract

The lower Chesapeake Bay, serves as the principal nursery ground for juvenile sandbar sharks (*Carcharhinus plumbeus*) in the western North Atlantic where they benefit from increased prey abundance. By occupying the Bay's lower salinity waters, juvenile sandbars also presumably benefit from reduced rates of predation by species such as dusky sharks (*Carcharhinus obscurus*), which do not enter the Bay.

In order to determine the extent to which sandbar sharks are able to use the Bay as a nursery area, their osmoregulatory abilities were quantified. Furthermore, limited numbers of dusky sharks were tested to examine the abilities of a congeneric, putative stenohaline elasmobranch for comparison. To accomplish this, juvenile sandbar and dusky sharks were kept in closed-system aquaria and the salinity incrementally reduced (33-27-20ppt and 25-17-12ppt) by addition of fresh water. The sharks were weighed and blood samples taken after experiencing each salinity for seven days. Haematocrit, haemoglobin, total protein, glucose, plasma osmotic pressure, Na^+, Cl^-, urea, and lactate concentrations were measured in each blood sample.

Significant decreases in osmotic pressure (982±5-417±4 mOsm), urea (457±6-66.1±5.2 mmol l^{-1}) and [Na^+] (268±2-232±2 mmol l^{-1}) were found for sandbars sharks between 33 and 12ppt. Dusky sharks however, were unable to cope with the change effectively and weren't tested beyond 20ppt. Their osmotic pressure concentration changes were 1019±3-657±4 mOsm, urea concentration changes were 387±17-177±9 mmol l^{-1} in and [Na^+] changes were 283±2-256±2 mmol l^{-1} in. These data imply that sandbar sharks are partial osmoregulators and ionoregulators coping with osmotic stress by maintaining themselves hyperosmotic and keeping high plasma ion levels in spite of increases in their extracellular water content and that dusky sharks are osmoconformers, incapable of surviving prolonged, significant osmotic burdens.

Key words: osmoregulation, urea, elasmobranch

1. Introduction

Sandbar (*Carcharhinus plumbeus*) and dusky (*Carcharhinus obscurus*) sharks both have worldwide distributions including populations along the North American eastern seaboard. Their ranges differ, however, in areas of lower salinity such as the Chesapeake and Delaware Bay estuaries. These congeneric elasmobranchs inhabit nearby summer nurseries and have overlapping distributions otherwise (Compagno 1984). However, sandbar sharks have been shown to venture into areas where the salinity is as low as 15 parts per thousand (ppt) (Grubbs 2007a), whereas dusky sharks do not (Musick and Colvocoresses 1986). Juvenile sandbar sharks use these estuarine habitats as nursery grounds and benefit from the high productivity and lower predator interaction (Medved and Marshall 1981, Musick et al. 1993, Grubbs 2007a). Dusky sharks' summer nurseries in the western North Atlantic include coastal areas from New Jersey to South Carolina (Castro 1993) but are rarely found in areas of reduced salinity (Musick et al. 1993).

Sandbar sharks are the most abundant and highly exploited shark species in the northwestern Atlantic with ~8,000 animals taken recreationally and ~1.9 million metric tons dry weight taken commercially in 2002 (NOAA 2004). These K-selected animals are slow growing, require 15-16 years to reach maturity (Sminkey and Musick 1995) and have very few large offspring (~9 per litter) (Springer, 1960, Lawler, 1976). As a result by 1991 the population levels had been reduced by 66% (Sminkey and Musick 1995) and even with management efforts, have not yet recovered to half their pre-exploitation levels. Dusky shark males mature at ~230 cm fork length (FL) around 19 years old and females at ~235 cm FL around 21 years old

(Natanson et al. 1995), with gestation periods estimated to be as long as 22-24 months by Branstetter and Burgess (1996). A possible year resting stage has been suggested for dusky sharks (Musick 1995, Branstetter and Burgess 1996, Romine 2004) and they have litters sizes of 3-14 pups. Based on the 2006 Stock Assessment of Dusky Sharks in the U.S. Atlantic and Gulf of Mexico (Cortes et al. 2006) and the models developed for this report it was determined that by International Union for the Conservation of Nature and Natural Resources (IUCN) criteria dusky sharks, which are already a prohibited species, would be classified as critically endangered. In order for these species populations to recover to viable fishery levels harvesting of mature animals must be conducted sustainably and immature animals allowed to reach sexual maturity. In comparing these two species it is important to consider that by using the Bay as refuge, the sandbar sharks, which are born considerably smaller (~60cm Branstetter and Burgess 1996) than the dusky sharks (~90cm Branstetter and Burgess 1996), have a chance to quickly attain sizes to match the dusky sharks without a major threat of predation.

In order to effectively manage a species that utilizes a unique nursery ground we must develop an understanding of how much of that habitat is available to the species in order to determine the most important areas for them. Musick et al. (1993) have shown that the Chesapeake Bay is a crucial nursery ground for sandbar sharks, but in order to protect these grounds correctly and devote resources most efficiently the osmoregulatory abilities must be quantified so that managers can establish accurate critical habitat possibilities.

Martin (2005) found that at least 162 extant elasmobranch species occur in

reduced salinity habitats. The change in ion concentration and osmotic pressure that results from a decrease in salinity requires that euryhaline species seeking to make that change effectively balance their osmolyte concentrations against the gradient between themselves and their environment or conform to the new medium. Without mechanisms to defend osmolyte concentrations elasmobranchs risk osmotic gains of water and diffusional losses of [Na^+] and [Cl^-] (Evans et al. 2004). Elasmobranch gills, with their large surface area, are highly permeable to water, including freshwater species like fresh water stingrays (*Potamotrygon* sp., Carrier and Evans 1973) and saltwater species such as the bluespotted ribbontail ray (*Taeniura lymma*, Tam et. al. 2003) and those that transit in between. Movement from marine to dilute waters leads to an increase in diffusive water influx, thus increasing the animals' body water content, which can cause changes in the blood osmotic pressure and ion concentrations due to haemodilution. Moreover, as haematocrit decreases, the blood's ability to deliver oxygen to the tissues can become impaired (Goldstein and Forster 1971). Elasmobranchs reduce their osmotic pressure primarily by decreasing urea, with additional reductions by Na^+ and Cl^- (Piermarini and Evans 1998, Smith 1931, Thorson et. al. 1973, Urist 62). Much effort has been placed on understanding how elasmobranchs manage these stresses. Cooper and Morris (1998a,b 2004a,b) showed that the Port Jackson shark, an elasmobranch with moderate osmoregulatory abilities, was able to tolerate salinities 50% less than full strength seawater.

Gaining a better understanding of how sandbar sharks cope with osmoregulatory stress and anticipating how much of estuarine habitats are utilized by these animals as a nursery ground will aid in supporting protection for their crucial

juvenile years in the nurseries. I hypothesized that the sandbar sharks would be able to survive reduced salinities as low as 60% of marine seawater and that the dusky sharks would be unable to osmoregulate at all and would be unable to manage a salinity reduction of 20% from marine. My objectives were to determine the osmoregulatory capabilities of two congeneric elasmobranch species whose juvenile forms inhabit nearby areas but are not both found in estuarine waters where the salinity is reduced. The objectives were met by monitoring the blood parameters of the animals following sequential dilutions of marine water to levels just below those found to be their respective environmental limits. The data presented here, in conjunction with field studies (Grubbs 2007 a, b) can be used to define maximum estuarine availability yearly for sandbar sharks, dependant upon Bay salinity profiles, and allow for more accurate protection to better allow for their populations to rebound.

2. **Materials and methods**

2.1.1 Animal Husbandry (sandbar sharks)

Sandbar sharks were caught during the summer months by hook and line from within the estuarine habitats formed by the barrier islands surrounding the Virginia (U.S.A.) eastern shore. Animals were held in a 47,300-l tank supplied with running seawater for up to 14 days until transfer to experimental pools, which were 3 m in diameter and 76 cm deep. Water in the experimental pools was initially supplied with filtered seawater (SW) (33ppt), which was recirculated and biofiltered, aerated, and UV sterilized. Temperature and salinity were measured with a field salinometer (YSI 30, Yellow Springs, OH), pH, nitrate, nitrite, ammonia, and phosphate levels were

measured every other day by test kit (Fastest STK1, Aquarium Systems, Mentor, OH). Alkalinity and copper levels were measured weekly (Fastest TF700, TF600 respectively). The sharks were fed Atlantic menhaden (*Brevoortia tyrannus*) and/or farm raised hybrid tilapia (*Oreochromis niloticus*) every other day until satiation. Animals were not fed for three days immediately prior to sampling.

2.1.2 Animal Husbandry (dusky sharks)

Dusky sharks were caught via longline immediately offshore of the Virginia eastern shore and were transported to the laboratory by shark tubes (appendix B) within two hours. They were held in the 47,300-l tank for three days prior to transfer to the experimental aquaria described above.

2.2 Experimental procedures

Both dusky and sandbar sharks were acclimated for seven days to seawater (SW) (33ppt). The salinity was then reduced over 48 hours by adding unchlorinated well water. The reduced salinity levels through the two experimental years for the sandbar sharks were: 27, 25, 20, 17, and 12ppt (Figure 1) or 80, 75, 60, 50, 36% of 33ppt. The dusky sharks experienced dilutions from marine to 80 and 60% SW. Control experiments were run in 2005 in which sandbar sharks were maintained at 100% SW for 25 days and sampled similarly to the experimental sharks (Figure 1). The sharks were then maintained for an additional seven days at the reduced salinity before 7 ml blood samples were taken by caudal puncture. Six-milliliter sub-samples of blood were immediately transferred to chilled heparinized syringes. The unheparinized sample was spun down and the plasma frozen within 10 minutes for later analysis of urea.

2.2 Blood and Plasma Sampling

For each sample, whole blood haemoglobin concentration ([Hb]) was determined by the cyanmethaemoglobin method (Dacie and Lewis 1984). Haematocrit (hct) was determined by centrifugation in microhaematocrit tubes. Red blood cell counts were made on blood samples (diluted 100:1 with shark plasma) using a Neubauer haemocytometer, with five microscope fields counted for each sample and averaged. Mean cell haemoglobin concentration was calculated from haemoglobin and haematocrit values (([Hb]x100)/[hct]).

Plasma was isolated by centrifugation. Plasma protein concentration was determined using a protein refractometer. Plasma osmotic pressure was measured with a vapor pressure osmometer (Wescor 5500), calibrated with 100, 290, 1000 mOsm standards weekly. Plasma samples for determination of urea levels were anticoagulant free as per assay kit instructions and frozen (-20^{o}C) for up to seven days before reading. Urea concentrations were measured spectrophotometrically using an assay test kit (Pointe Scientific Inc. B7550, Canton, MI). Plasma samples to be used for lactate assays were deprotienated with 6% perchloric acid and frozen (-20^{o}C) for up to seven days. Lactate levels were subsequently measured with an assay test kit (Trinity Biotec No. 735, Bray, Ireland). Values for [Na^{+}], [Cl^{-}], and glucose were analyzed with an iStat Portable Clinical Analyzer (PCA) (Abbott, Abbott Park, IL). The iStat PCA is an FDA approved microprocessor-controlled instrument commonly used by the medical industry capable of reading whole blood and plasma samples and determining concentrations of a large suite of parameters. Prior to use,

iStat readings were compared independently (South Bend Medical Foundation) with traditional techniques on shark blood and plasma for all of the measured factors to ensure accuracy.

2.3 Data Analysis

Statistical analyses were performed using R (2.3) for OS X (10.4.7). Data were square root transformed to stabilize the variance and improve normality in the residuals. Many of the dependent variables were correlated; for example, a change in urea concentration has an effect on osmotic pressure. Principal component analysis was used to reduce the dimensionality and to obtain orthogonal linear combinations of the original variables.

Independent variables were salinity, which was treated as a continuous variable and weight of each shark during each experiment. Mixed effects models for each dependent variable and the principal components were fit to the data using restricted maximum likelihood (REML) to estimate the added variance due to random effects: the repeated measures of each shark and the two years over which the research was done. The model used was: variable = weight + sal + sal^2 + year + shark in year + error. The repeated measures of sharks were a result of sampling each animal over the three salinity treatments. The experiments were carried out over two years due to logistical constraints. As a result, unmeasured variables probably contributed some variance across the two years, which should be included in the analysis. Most of the dependent variables were best fit by treating salinity as a second-degree orthogonal polynomial. Linear and quadratic effects of decreased salinity was estimated for each variable

An analysis of variance was done on the mixed effects models to determine if salinity changes contributed to the variance of the dependent variables. Weight of each shark at the time of testing was included as a covariate in all models. T-tests were used to test for differences between control and experimental animals.

3. Results

3.1. Animal Condition

Experiments to determine blood parameters under control conditions (100% SW) and to determine if captivity would have significant effects on the animals that survived transfer and handling stress, resulted in the conclusion that the animals could survive captivity without incident and that captivity played no significant part in the concentration of the blood parameters measured. Control experiments were done on sandbar sharks only.

Sandbar and dusky sharks were successfully acclimated to captivity and were feeding within two days of entering the experimental aquaria. In 2005, there were two mortalities in the first two days after transfer, during the control experiment and a single mortality in the final week while the animals were at 60% SW. In 2006 there was a single mortality in the second week, at 50% SW, and three during the final week at 36% SW. The remaining sandbar sharks were incrementally brought back to full strength SW and returned to the holding tank. The animals in 2005 all survived this transfer, whereas in 2006 they all died within two days of being transferred. One dusky shark died in the final week of experimentation at 80% SW, the other two died in the third week at 60% SW, shortly after being sampled, which was done two days ahead of schedule due to behavioral cues indicating high stress.

In 2005 the sandbar sharks showed an average weight increase of 14.4% from 100 to 60% SW and in 2006 showed a 9.6% increase from 75 to 36% SW. Sandbar sharks in control experiments did not have any significant weight changes. The dusky sharks had a 3.5% increase through the experiment from 100 to 60% SW.

3.2. Whole Blood and Plasma

Mean values of all measured parameters resulting from the sequential reductions in salinity are given in Tables 1 and 2 for sandbar and dusky sharks, respectively. The models fit to the measurement data take into account the multiple years of data taken and the repeated sampling of sharks throughout the experiment, and consider possible trends that exist. Only sandbar shark data was modeled because aquarium size restricted keeping more than one dusky shark per pool and sample size didn't remain high enough for statistical measure, however general comparisons will be made.

No statistical difference existed between sandbar sharks at 100% SW in the experimental treatment and sharks at control treatments except for chloride and total protein concentrations. The unexpected difference in chloride is a result of high variability in the experimental sharks, which ranged 160-212 mmol l^{-1} that wasn't seen in the controls, which only ranged 204-230 mmol l^{-1}. Similar variability differences were seen in total protein concentration, which ranged from 7.6 -7.9g ml^{-1} for control animals and 6.1-7.5 g ml^{-1} in the experimental sharks at 100% SW,

Salinity contributed a significant component of variance ($P<0.05$) in all of the reported regression results.

Experiments were conducted in decreasing salinity increments, however

graphs and discussion will be related to increasing salinity trends. Haemoglobin showed a significant ($P>0.05$) increasing linear trend with increasing salinity through the two years (Table 5). Haematocrit also showed a significant increasing linear relationship (Table 5). This suggests that the animals were likely experiencing haemodilution at lower salinities, most likely resulting from water uptake. Total protein concentration had a significant increasing linear and quadratic trend with increased salinity (Table 5).

There were also significant influences of the reductions in salinity on measured osmolytes. The osmotic pressure regression showed a highly significant linear and quadratic increasing trend (Table 5) as both species tracked the change in salinity but the degree to which they were able to remain hyperosmotic was different in comparison to each other as well as other species (Figure 3). The urea concentration had a high linear increase with increased salinity (Table 5) which is not surprising because it is the primary osmolyte elasmobranchs use and as environmental osmotic pressure changes with salinity the animals adjust their osmotic pressure by controlling urea concentration to reduce the differential gradient between the ambient conditions and themselves. Sodium concentration had high linear and quadratic increasing trend (Table 5). Chloride did not have a significant change with increased salinity (Table 5).

Glucose concentration varied (48.0-59.8 mg dl^{-1}) but still had a significant increasing linear trend (Table 5), and that range is likely attributable to the varying degrees of hunger exhibited by the animals. Lactate was only elevated in instances where sampling exceeded one minute.

4. Discussion

Elasmobranchs maintain themselves hyperosmotic to their environment (Hochachka and Somero 2002) primarily by keeping high levels of urea and methylamines (trimethylamine oxide [TMAO], sarcosine, and betaine), in concentrations within a general ratio of 2:1(Yancey and Somero 1979, Weber 1983), urea to methylamines. The TMAO serves as an osmolyte and also serves to counteract the denaturing effects of the high urea concentrations on proteins (Hochachka and Somero 2002). The gills are the sites of net salt uptake (Piermarini et al. 2002, Wood et al. 2002), whereas the rectal gland is the major site of salt excretion and the kidney maintains the high urea and methylamine concentration by reabsorbtion and to some lesser extent is able to reabsorb sodium and chloride (Evans et al. 2004).

Cooper and Morris (1998) developed a scale to reflect osmo/iono-regulatory abilities based on the ratio of osmotic or ionic change, as a percentage, in seawater over a salinity decrease to the percent change of that parameter in the animal, which can be used to judge regulatory ability. A ratio of 2 is baseline of an osmoregulating species (ie. *Dasyatis sabina,* Atlantic stingray), >1 but <2 a partial osmoregulator, a generally euryhaline species with preference for high to intermediate salinity 1 is a perfect osmoconformer, <1 a strictly marine species (ie. *Squalas acanthis,* spiney dogfish). Calculation of the percent change in SW was made with reference values of salinity in seawater (35ppt being full strength with 468.96 mmol l^{-1} of Na^+ and 545.88 mmol l^{-1} of Cl^- [Pilson 1998]). Truly euryhaline species like the bull shark, *Carcharhinus leucas* (Thorson et. al. 1973) (ratio 3.3 [OP], 6.5 [Na^+]) which both

osmoregulate and ionoregulate and the Atlantic stingray, *Dasyatis sabina* (Piermarini and Evans 1998) (ratio 2 [OP], 1.8 [Na^+]) (Table 4) which osmoregulates and has high partial ionoregulatory ability are capable of maintaining their equilibrium in both fresh and marine waters, whereas less euryhaline species such as the Port Jackson shark, *Heterodontus portusjacksoni* (ratio 1.2 [OP], 1.1 [Na^+]) and the common stingaree, *Trygonoptera testacea* (ratio 0.8 [OP], 2.4 [Na^+]) (Cooper and Morris 1998a) (Table 4) are limited partial osmo/iono-regulators but are able to survive forays into lowered salinities but are limited by temporal exposure and their minimum salinity tolerance.

The data suggest sandbar sharks are euryhaline ionoregulators and partial osmoregulators and that they are capable of tolerating limited seawater dilutions but most certainly do not regulate both their internal osmotic and ionic states as well as the most euryhaline elasmobranchs. Dusky sharks, in contrast, are stenohaline osmoconformers incapable of surviving in dilute mediums.

In comparison to the best elasmobranch osmoregulators (bull shark and Atlantic stingray) (Figure 3) sandbar and dusky sharks are clearly incapable of maintaining internal osmotic pressure to the same degree, though they do continue to remain hyperosmotic to their environment. Euryhaline elasmobranch osmoregulators like the Atlantic stingray are able to regulate an osmotic change from FW to SW with only a 40% change in internal osmotic pressure (Piermarini and Evans 1998) and the bull shark can handle the same gradient with only a 30% osmotic pressure change (Thorson et al. 1973). The Port Jackson shark and common stingaree, partial osmoregulators, had a 44% and 47% decrease respectively in a dilution of 50% SW.

The spiny dogfish, a stenohaline osmoconforming elasmobranch species showed a 32% decrease in plasma osmotic pressure in 72 hours when marine salinity was diluted by only 30%. The euryhaline sandbar sharks, demonstrated partial osmoregulatory abilities with osmotic pressure changes of 58% from full strength SW to 36% SW. The stenohaline dusky sharks had a 36% decrease from full strength SW to 60% SW. Furthermore, the sandbar sharks ratio was ~1.5 [OP] in 60% SW (Table 4) and reduced to ~0.58 in 36% SW and the dusky sharks was ~0.80 [OP] in 60% SW (Table 4) which support the comparisons made here. Though the sandbar sharks are able to osmoregulate to 60% SW their abilities are limited and as salinity reductions continue their abilities to regulate diminish.

Change in the sandbar shark primary osmolyte, urea, reflected the change in SW strength (Figure 2B). The sandbar sharks urea concentrations in full strength SW were higher than values reported for bull sharks (Pillans et al. 2005) and Atlantic stingrays (DeVlaming and Sage 1973) but at 50% SW were lower than Atlantic stingrays (Piermarini and Evans 1998) and bull sharks in FW (Pillans et al. 2005). With an overall urea concentration change of 75% in a 50% SW dilution, the sandbar sharks had a greater urea loss than the common stingaree and Port Jackson shark which had a 50% and 60% decrease, respectively (Cooper and Morris 1998a) but kept far higher urea concentrations than the spiny dogfish which had a 70% reduction in SW diluted by 30% (Forster et al 1972). When the sandbar sharks were reduced to 36% SW they showed an 86% decrease in urea, comparable to the values found by Pillans et al. (2005) for the bull shark, whose urea concentrations reduced by 95% from SW to FW. The dusky sharks showed the ability to maintain urea

concentrations better than sandbar sharks and at 60% SW had approximately 20 mmol l^{-1} more urea. This may be in part due to the fact that they didn't keep concentrations of other osmolytes as high.

Sodium ion concentration decreases were marked in sandbar sharks throughout the experiments (13.4%) from 100%-36%, but not to the extent of the Port Jackson shark after one week at 50% SW (43%) or than the common stingaree (17%) which demonstrated partial ionoregulating abilities (Cooper and Morris 1998a). The sandbar sharks also showed better ion regulation than the spiny dogfish which had a 12% decrease in $[Na^+]$ and a 10% decrease in $[Cl^-]$ in twenty four hours over a 25% salinity reduction from full strength SW (Schmidt-Nielson et al. 1972). The sandbar sharks $[Cl^-]$ showed a slight increase over the first few increments and then return to values equal to those at full strength in the lowest salinity dilutions. A change similar to that of $[Na^+]$ in the $[Cl^-]$ trend was expected for the sandbar sharks, however it is possible that the sharks manage them differently and selectively sequester $[Cl^-]$. *Leucoraja ocellata*, the winter skate, has been suggested to differentially regulate individual plasma solutes because of the degree to which changes in [urea], [TMAO], $[Na^+]$, and $[Cl^-]$ changed were different, although $[Na^+]$ and $[Cl^-]$ were similar (Sulikowski et al. 2003). Sandbar sharks have ionoregulatory capability, possibly helping to allow for urea concentrations that were consistently lower than the Atlantic stingray and bull shark at salinities in which all three species are found regularly (DeVlaming and Sage 1973, Pillans et al. 2005). Elasmobranch osmoregulators generally exhibit ionoregulatory abilities with $[Na^+]$ and $[Cl^-]$ ratios >2, with the exception of the Atlantic stingray, which conserves plasma urea instead of $[Na^+]$ and

[Cl^-] (Cooper and Morris 1998a). By maintaining high [Na^+] and [Cl^-] levels these animals likely retain ion distributions intra- and extra-cellularly in proper ratios to allow for required ion balances for nervous and muscle system function and for the function of all excitable cells. The differential management of these ions was documented in dusky sharks (Cliff and Thurman 1984), where, after a 24-hour recovery from stress [Cl^-] concentrations were on the order of 25 mmol l^{-1} higher than [Na^+]. The dusky sharks didn't show management to that degree in these experiments but were able to maintain constant [Cl^-] concentrations. Their [Na^+] concentrations declined more dramatically than the sandbar sharks, though they did have higher concentration at 100% those values decreased substantially at each decrease in salinity. Furthermore, the sandbar shark [Na^+] ratio was ~7.0 in 60% SW and ~5.3 in 36% SW (Table 4) suggestive of the same decrease in abilities as found in the osmotic pressure ratios but different in that they retain [Na^+] regulatory abilities, though reduced in more dilute SW concentrations. The dusky sharks ratios were ~4.26 [Na^+] in 60% SW (Table 4), less than that of the sandbar sharks but similar in that even with substantial reductions in osmotic pressure the animals continue to retain [Na^+] regulatory abilities.

Sandbar sharks demonstrated a limited ability to counteract the water influx and showed weight gains that could not be attributed solely to feeding (14.4% in 2005 and 9.6% in 2006). Their total protein concentration showed a significant decrease of 40%, similar to that of the euryhaline bull shark that showed a difference between FW and SW conditions of 39% (Thorson et al. 1973). The significant protein concentration decline is most likely due to dilution of plasma and interstitial fluids,

presuming capillaries of elasmobranchs are as permeable as those of teleosts (Olson and Farrell 2006). Furthermore the sandbar sharks maintained almost double the total protein concentrations as the bull and bonnethead sharks (Thorson et al. 1973 and Harms et al. 2002 respectively) regardless of salinity. The dusky sharks had a more pronounced decrease than the sandbar sharks, due in part to their total protein being at higher concentrations in 100% SW and they were lower than sandbar sharks in 60% SW.

Considering the significant decreases in haematocrit and haemoglobin concentration (Table 1), the sandbar sharks experienced haemodilution, which likely resulted in impaired blood respiratory capability and decreased oxygen flow to the cells. The dusky sharks showed similar decreasing trends in haematocrit and haemoglobin but began and ended at lower overall concentrations (Table 2). The sandbar sharks were able to maintain a higher blood respiratory capability, even if impaired, than the dusky sharks, supporting evidence that sandbar sharks are able to regulate osmotic burdens to a degree higher than a stenohaline osmoconforming elasmobranch. The lack of change in sandbar shark MCHC (Table 5) and the decline of haematocrit, haemoglobin and protein concentration suggests that there are less red blood cells present in the plasma as oppose to possible red blood cell shrinkage. Piermarini and Evans (1998) showed that bull sharks had no significant haematocrit change, demonstrating that they could completely counteract any water influx as the salinity gradient increased. Cooper and Morris (1998a) showed the Port Jackson shark and common stingaree couldn't withstand increased salinity gradients as both species did have haematocrit decreases, however all values returned to control levels

after one week in SW. The sandbar sharks demonstrated abilities similar to the Port Jackson shark and common stingaree, which experienced haemodilution at non-fatal levels and were able to return to marine salinities without detriment at salinity reductions less than half of marine.

Lactate concentrations in sandbar sharks did not show any major increase except in cases where sample time exceeded one minute (Table 1) and didn't approach values of stressed sandbar sharks presented in Brill et al. (Submitted) which were 34.9 ±0.5 mg dl^{-1}. Neither captivity nor average sampling time stressed these animals greatly. The dusky sharks however showed increasing lactate concentrations throughout experimentation, suggestive that the change in environmental salinity resulted in increased stress to them.

The sandbar shark ability to effectively maintain an internal balance against osmotic stress and return to full strength SW in 2005 suggests that the animals are euryhaline and should be able to transit easily into Bay waters as low as 60% SW regularly. Forays into waters lower in salinity than that are most likely brief, suggested by the very low catch rates on VIMS' longline survey (Grubbs 2007) as well as the animals' inability to reacclimate to full strength SW in 2006 as the animals in the previous year had. Although dusky sharks are a congeneric species, it is unsurprising that all of them were unable to survive the salinity change because they are not caught in the Bay by the VIMS longline survey (Musick et al. 1993) and reports of them are likely misidentification of sandbar sharks. The sandbar shark, by using the Chesapeake Bay as a nursery also effectively avoids bull sharks, which can enter environments as dilute as fresh water but whose geographical range is

temperature limited and doesn't usually extend as far north as the Bay. However, in more southerly regions such as the Gulf of Mexico adult bull sharks are major predators of juvenile sandbar sharks (Springer 1960). By being able to omsoregulate to limited degrees, the euryhaline sandbar shark has evolved a physiological capability to segregate themselves at smaller sizes from potential stenohaline predators, which cannot effectively osmoregulate. Sandbar sharks then attain larger sizes before leaving the primary nursery in the fall of the year (Musick and Colvocoresses 1986).

5. Conclusions

As sandbar sharks only regularly experience reduced salinities during their juvenile years it is understandable that they don't require the development of any complex mechanisms, biochemical or physical, to manage the osmotic burden. In comparison with other elasmobranch species, the sandbar sharks maintain some of the measured parameters at levels reflective of regulators, some as partial regulators and some bordering conformers over the salinity gradients that they were exposed to. They effectively tolerate limited estuarine penetration because of the substantial benefits afforded within, but clearly are not as euryhaline tolerant as species like the bull shark or Atlantic stingray. Neither sandbar nor dusky sharks are truly euryhaline primarily because of significant haemodilution and only moderate hyperosmotic maintenance at reduced environmental salinity in comparison to the most euryhaline elasmobranchs (Fig. 3). Sandbar sharks are moderately euryhaline ionoregulators and partial osmoregulators and dusky sharks are stenohaline osmoconformers with ionoregulatory abilities.

Table 1. Blood and plasma state of sharks at experimental salinities.

	Sandbar (*Carcharhinus plumbeus*)					
	100%SW [9]	80%SW [9]	75%SW [9]	60%SW [8]	50%SW [8]	36%SW [4]
*[A]Haematocrit (%)	17.63 ± 0.52	18.30 ± 0.50	15.74 ± 0.32	14.79 ± 0.61	15.75 ± 1.14	14.42 ± 0.54
*[A]Haemoglobin (g dl^{-1})	3.48 ± 0.09	3.59 ± 0.13	3.40 ± 0.14	3.05 ± 0.13	3.03 ± 0.22	2.69 ± 0.04
*#[A]Total Protein (g/100ml)	6.81 ± 0.15	6.72 ± 0.14	5.84 ± 0.15	4.94 ± 0.18	5.13 ± 0.23	4.10 ± 0.30
*[A]Glucose (mg dl^{-1})	52.33 ± 2.10	59.78 ± 0.91	49.50 ± 1.63	48.00 ± 0.10	52.38 ± 1.71	58.13 ± 2.62
Mean Cell Hemoglobin (g l^{-1})	19.79 ± 1.23	21.54 ± 1.04	19.61 ± 1.77	20.79 ± 2.74	19.28 ± 1.26	18.77 ± 1.50
Lactate (mmol l^{-1})	0.21 ± 0.05	0.22 ± 0.03	0.24 ± 0.04	1.16 ± 0.78	0.39 ± 0.12	0.15 ± 0.03

Table 1.

The values of the given concentrations of parameters measured from whole blood and plasma for *C. plumbeus*. All values are given as means ±SEM. The *asterisk* denotes parameters whose model showed a significant linear trend as a result of salinity. The *pound sign* denotes parameters whose model showed a significant quadratic trend. The letter *A* denotes parameters whose ANOVA test was significant. Significance was determined at the 95% ($p<0.05$) confidence level. All significance testing was done for *C. plumbeus* only. Numbers in brackets [] denote sample size.

Table 2. Blood and plasma state of sharks at experimental salinities.

	Dusky (*Carcharhinus obscurus*)		
	100%SW [3]	80%SW [2]	60%SW [2]
Hct (%)	17.0 ± 0.42	16.4 ± 1.82	12.3 ± 3.31
Hb (g dl^{-1})	2.88 ± 0.18	2.58 ± 0.17	2.06 ± 0.55
Total Protein (g/100ml)	7.13 ± 0.17	5.97 ± 0.12	4.15 ± 0.65
Glucose (mg dl^{-1})	113 ± 5.03	95.7 ± 8.42	106 (--)
Mean Cell Hemoglobin (g l^{-1})	16.94 ± 0.81	15.96 ± 1.71	16.75 ± 0.05
Lactate (mmol l^{-1})	0.06 ± 0.01	0.92 ± 0.52	1.11 ± 0.94

Table 2.

The values of the given concentrations of parameters measured from whole blood and plasma for *C. obscurus*. All values are given as means ±SEM. Numbers in brackets [] denote sample size.

Table 3. Osmotic, ionic, and blood state of sharks at control salinity (100%SW)

	Sandbar (*Carcharhinus plumbeus*)		
	Week 1 [7]	Week 2 [7]	Week 3 [7]
Hct (%)	21.90 ± 0.93	19.48 ± 0.97	19.19 ± 0.73
Hb ($g\ dl^{-1}$)	3.55 ± 0.13	3.81 ± 0.16	3.84 ± 0.15
Total Protein (g/100ml)	7.91 ± 0.12	7.54 ± 0.10	7.61 ± 0.13
Glucose ($mg\ dl^{-1}$)	61.0 ± 2.27	55.57 ± 3.79	56.37 ± 2.90
Mean Cell Hemoglobin ($g\ l^{-1}$)	19.33 ± 0.76	19.67 ± 0.65	20.02 ± 0.44
Lactate ($mmol\ l^{-1}$)	0.29 ± 0.06	0.18 ± 0.03	0.34 ± 0.17
Osmotic Pressure (mOsm)	1015.45 ± 4.83	1013.88 ± 6.62	1007.55 ± 3.20
Urea ($mmol\ l^{-1}$)	467.93 ± 15.81	472.94 ± 11.14	476.68 ± 12.27
Na ($mmol\ l^{-1}$)	292.57 ± 2.38	288.0 ± 2.83	298.57 ± 2.98
Cl ($mmol\ l^{-1}$)	219.43 ± 3.14	217.14 ± 2.65	226.0 ± 3.12

Table 3.

The values of parameters measured from whole blood and plasma for *C. plumbeus* for the full strength control salinity. All values are given as means ±SEM. Numbers in brackets [] denote sample size.

Table 4. Ratios calculated for plasma parameters

	Osmotic Pressure	Sodium
Sandbar Shark (*C. plumbeus*)		
100-80 (168 h)	**1.5**	***
80-60 (168 h)	**1.5**	**7.0**
75-50 (168 h)	**0.8**	**3.3**
50-36 (168 h)	**0.6**	**5.3**
Dusky Shark (*C. obscurus*)		
100-80 (168 h)	**1.4**	**5.0**
80-60 (168 h)	**0.8**	**4.3**
Bull Shark (*C. leucas*)		
100-0 (field)	**[e]3.3**	**[e]6.5**
Atlantic Stingray (*D. sabina*)		
100-55 (114 h)	**[e]2**	**[e]1.8**
Port Jackson Shark (*H. portjacksoni*)		
75-50 (168 h)	**1.2**	**[e]1.2**
Common Stingaree (*T. testacae*)		
75-50 (168 h)	**0.8**	**2.4**

Table 4.

Table 4. Ratios calculated of osmo/iono-regulatory capabilities for elasmobranch species. Sandbars shark sodium ratio is not calculated as no change was evident from 100-80% SW. Superscript e (°) represents values estimated from Cooper and Morris 1998a.

Table 5 Regression table of measured variables in experimental sandbar sharks

Haematocrit					
		Coefficient	Std.Error	t-value	p-value
	Intercept	15.2	1.9	7.6	0.0
	Covariate (Weight)	0.52	0.72	0.72	0.48
	Linear (Salinity)	11.2	2.0	5.6	0.0
	Quadratic (Salinity)	-0.93	1.55	-0.59	0.55
Haemoglobin					
		Coefficient	Std.Error	t-value	p-value
	Intercept	2.7	0.31	8.8	0.0
	Covariate (Weight)	0.25	0.13	1.9	0.07
	Linear (Salinity)	2.2	0.45	4.9	0.0
	Quadratic (Salinity)	-0.69	0.36	-1.9	0.07
Mean Cell Haemoglobin					
		Coefficient	Std.Error	t-value	p-value
	Intercept	18.9	1.8	10.3	0.0
	Covariate (Weight)	0.43	0.68	0.62	0.54
	Linear (Salinity)	0.49	2.0	0.24	0.81
	Quadratic (Salinity)	-3.3	1.6	-2.1	0.04
Red Blood Cell Count					
		Coefficient	Std.Error	t-value	p-value
	Intercept	230995.8	31582.8	7.3	0.0
	Covariate (Weight)	-16553.2	13137.6	-1.3	0.22
	Linear (Salinity)	-33806.8	50474.6	-0.67	0.51
	Quadratic (Salinity)	-19324.2	42390.7	-0.46	0.65
Total Protein					
		Coefficient	Std.Error	t-value	p-value
	Intercept	5.4	0.7	7.7	0.0
	Covariate (Weight)	0.15	0.23	0.67	0.51
	Linear (Salinity)	7.8	0.46	16.9	0.0
	Quadratic (Salinity)	-1.1	0.34	-3.3	0.0
Osmotic Pressure					
		Coefficient	Std.Error	t-value	p-value
	Intercept	856.3	32.9	26	0.0
	Covariate (Weight)	-40.8	12.9	-3.2	0.0
	Linear (Salinity)	1024	48.6	21.1	0.0
	Quadratic (Salinity)	-192.5	39.8	-4.8	0.0
Sodium					
		Coefficient	Std.Error	t-value	p-value
	Intercept	255.9	8.7	29.4	0.0
	Covariate (Weight)	1.9	3.6	0.54	0.59
	Linear (Salinity)	75.0	13.2	5.7	0.0
	Quadratic (Salinity)	-41.5	10.8	-3.8	0.0
Chloride					
		Coefficient	Std.Error	t-value	p-value
	Intercept	194.6	13.3	14.7	0.0
	Covariate (Weight)	-0.81	5.5	-0.15	0.88
	Linear (Salinity)	13.3	22.2	0.59	0.55
	Quadratic (Salinity)	-19	19.2	-0.99	0.33
Glucose					
		Coefficient	Std.Error	t-value	p-value
	Intercept	48.8	5.6	8.8	0.0
	Covariate (Weight)	1.9	1.7	1.2	0.24
	Linear (Salinity)	15.9	6.1	2.6	0.02
	Quadratic (Salinity)	3.9	4.9	0.8	0.43
Urea					
		Coefficient	Std.Error	t-value	p-value
	Intercept	253.2	40.8	6.2	0.0
	Covariate (Weight)	1.1	15.9	0.07	0.95
	Linear (Salinity)	910.2	58.1	15.7	0.0
	Quadratic (Salinity)	86.4	47.2	1.8	0.08

Table 5. Regression table of measured variables in sharks subject to dilute salinities. Coefficient, standard error, t-value, p-value are presented. Significance was determined at the 95% ($p<0.05$) confidence level.

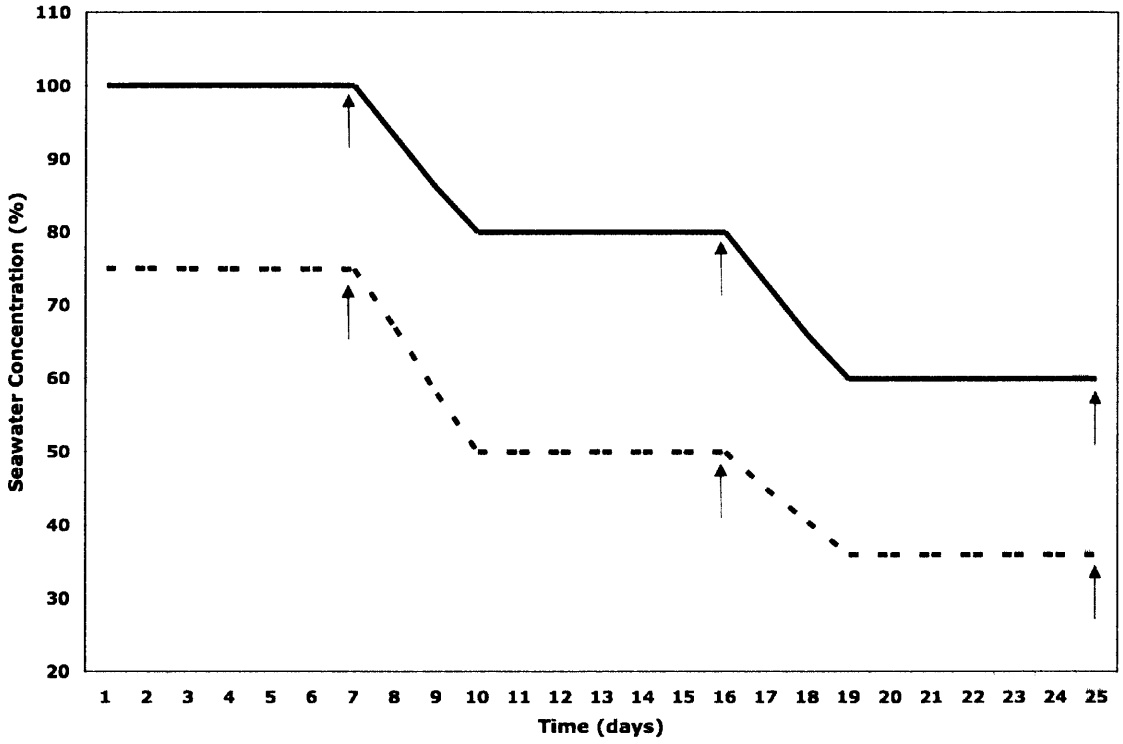


Figure 1

The sequential reduction of salinity throughout the experiments in 2005 and 2006. (---) Represents the 2006 regime, (—) represents the 2005 regime. Arrows indicate sampling days.

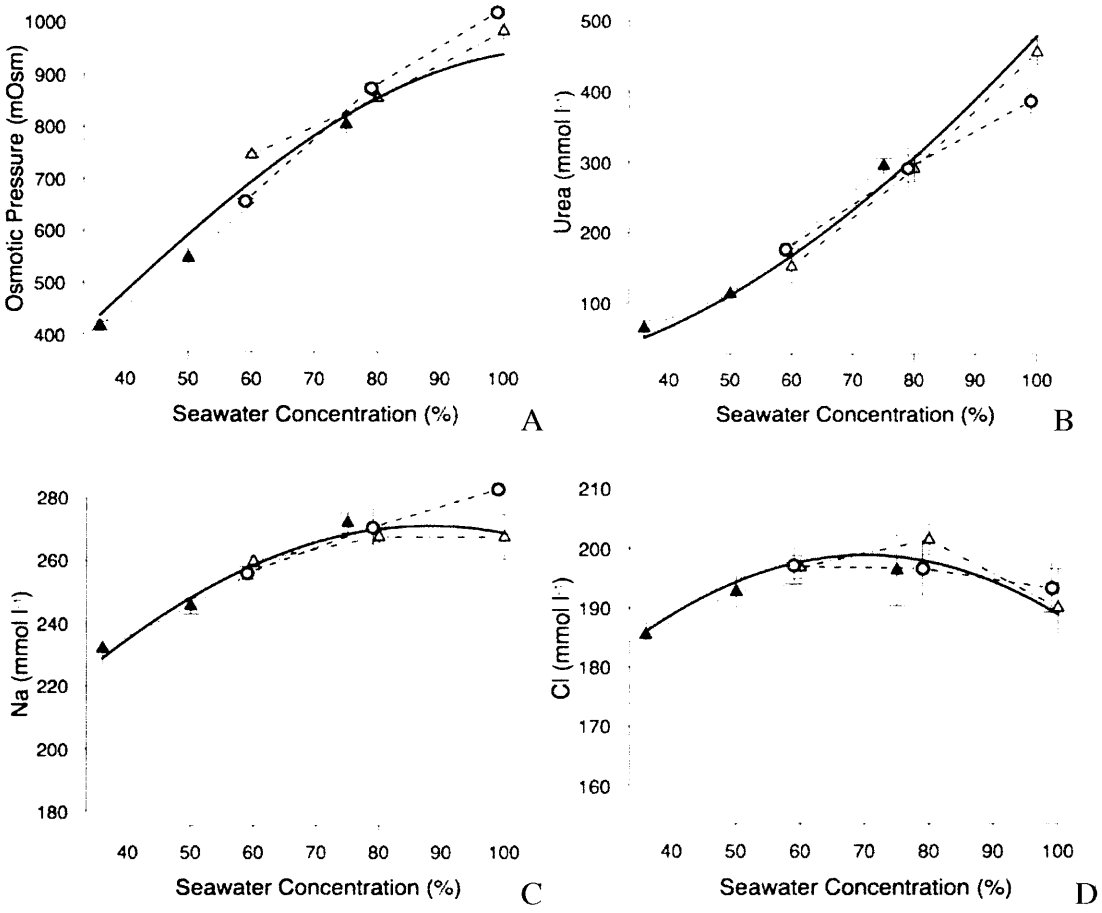


Figure 2

(A) Plasma osmotic pressure (B) urea (C) sodium (D) chloride concentrations as a function of salinity in two years of experimentation. Sandbar shark data in 2005 is represented by (Δ) and 2006 by (▲). Dusky shark data is represented by (O) and only error bars are present by treatment. Bold lines designate model fit. X-axis values where two data points exist are offset.

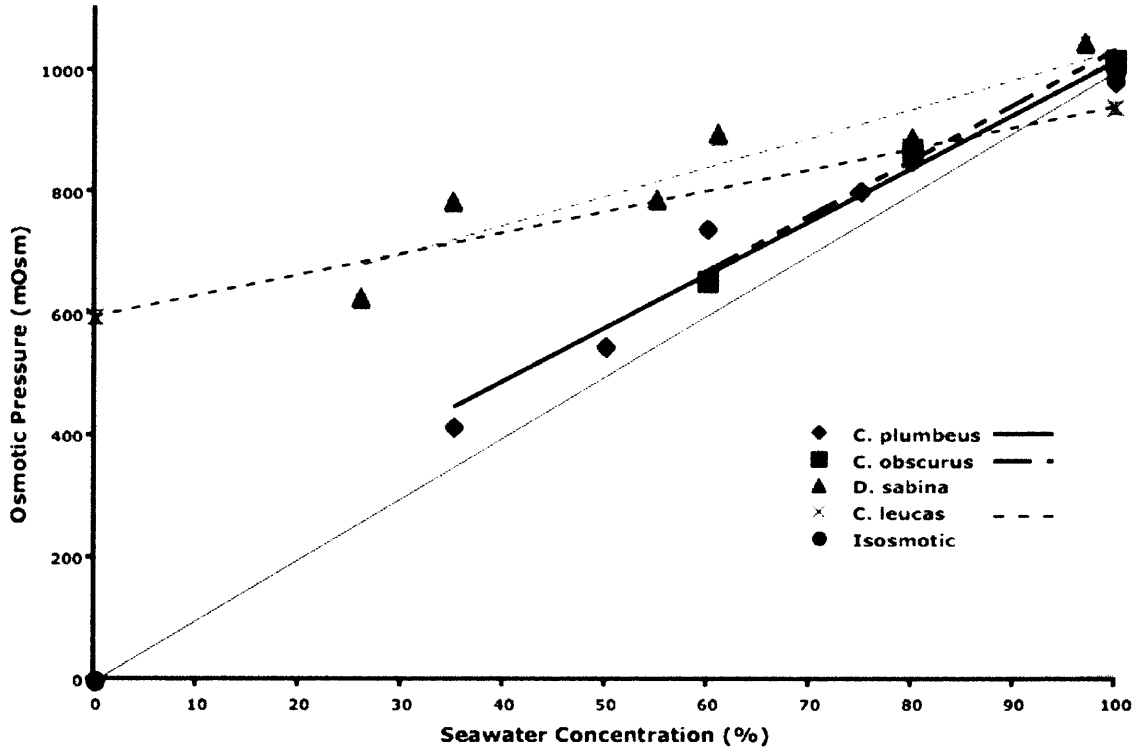


Figure 3

The osmotic pressure changes in response to salinity change in: C. plumbeus (◆) (this study), C. obscurus (■) (this study), D. sabina (▲) (DeVlaming and Sage 1973), C. leucas (×) (Thorson et al. 1973), Isosmotic (●). Standard error bars are presented but in many cases are too small to see beyond icons.

APPENDICES

Life History

Sandbar Shark (*Carcharhinus plumbeus*)

Carcharhinus plumbeus has a worldwide distribution, including populations in the Pacific along the Asian coast, the eastern coast of Australia, and Hawaii; in the Indian Ocean along Australia's western coast and Africa's eastern coast; and in the Atlantic along the western coasts of Africa and Europe, the eastern coast of North and South America as well as in the Caribbean (Springer 1960, Sminkey and Musick 1995). In the western North Atlantic sandbar sharks distributions in summer months span from Cape Cod, MA to the Yucatan peninsula and in winter from the Carolina coasts to the Yucatan (Bigelow and Schroeder 1948, Sminkey and Musick 1995). Sandbar sharks inhabit water depths ranging from the surface to ~280m (Compagno 2005), principally being found at depths from 20-55m.

Sandbar sharks make use of inland bays and shallow water habitats as nursery grounds, the primary nursery ground for the Northwest Atlantic stocks being the Chesapeake Bay. The Delaware Bay serves as a secondary nursery area. Sandbar sharks have viviparous, yolk sac placental reproduction, having between 10 and 12 pups in a litter (Musick et. al. 1993), dependant upon the female's size and resultant capability to support the pups. Gestation periods are 8-12 months, and females will pup approximately every other year allowing time to restore fat reserves. Size at maturity ranges between 140-180cm TL, from 15-16 years of age (Sminkey and Musick 1995), with a maximum size of ~240cm TL (Compagno 2005).

Sandbar sharks primarily feed on fishes and epibenthic crustaceans (Springer

1960) as well as elasmobranchs and cephalopods (Ellis 2003). The major predators of sandbar sharks are larger shark species, and predation is highest on the younger age classes.

Dusky Shark, (*Carcharhinus obscurus*)

The dusky shark, *Carcharhinus obscurus,* has a worldwide distribution, being found in warm temperate and tropical waters in both coastal and pelagic zones. Their range in the western North Atlantic spans from southern New England to the Caribbean and the Gulf of Mexico and as far south as in waters off of Brazil. Tagging work by Kohler et al. (1998) showed their range to extend to the Yucatan. Their depth ranges from the surf zone to a maximum of 400m (Compagno 2005). The dusky shark has been shown to avoid areas of reduced salinities such as estuaries (Compagno 1984, Musick et al. 1993). Dusky shark populations also undertake large localized migrations, in the western North Atlantic they move north towards New England and back south towards the Caribbean in winter (Musick and Colvocoresses 1986) and adults have been shown to make longer journeys than juvenile and neonates, within their range. Juveniles tend to inhabit coastal zones of high productivity (Castro 1993).

The dusky shark reaches a maximum size at 360cm TL (Castro 1993). In the western North Atlantic males reach sexual maturity at ~230cm FL around 19 year old and females at ~235cm FL around 21 years old (Natanson et al. 1995). Their reproductive methods have not yet been fully described. Gestation has been estimated to be as long as 22-24 months by Branstetter and Burgess (1996) and a

possible year resting stage has been suggested (Musick 1995, Branstetter and Burgess 1996). Dusky sharks are viviparous with 3-14 pups per litter.

Dusky sharks feed on flatfishes, groupers, jacks, other elasmobranchs, invertebrates and varied reef fishes (Castro 1983). Diet studies by Gelsleichter et al. (1998) showed that the index of relative importance, expressed as percentage (%IRI), was 85% teleost, 11.1% elasmobranch, 3% crustacean, 0.1% *L. polyphemus*, <0.1%mollusk and <0.1% turtle. Dusky sharks major predators are larger elasmobranchs.

Extended Materials and Methods

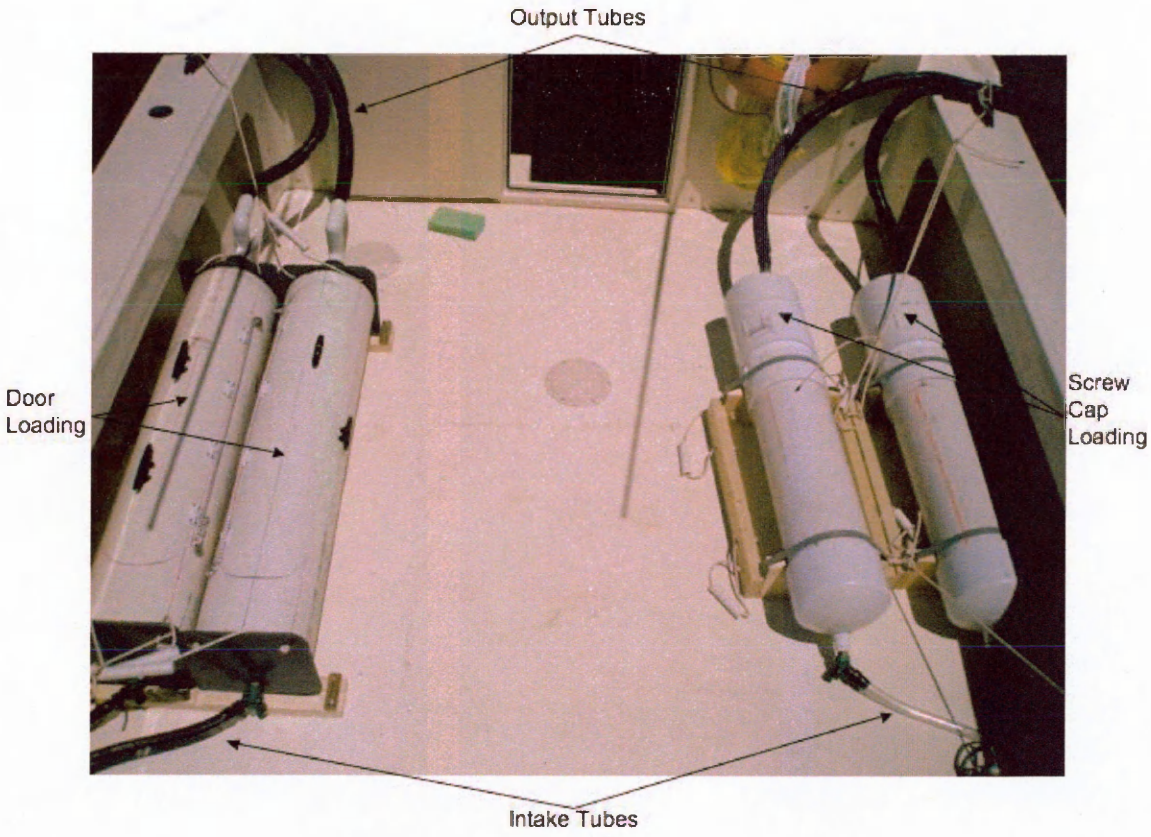
Capture Considerations

Due to reports of limited increasing catch rates in past years and high cost per fishing attempt it was decided that three trips would be designated for dusky shark capture. Fishing commenced after reports of dusky sharks being caught on the VIMS longline cruise began to come in and Joshua Smith reported dusky sharks being caught on the Eastern Shore during longlining being conducted for his research. This plan fit budget requirements because fishing for this project didn't being until several reports of the animal in the area had been received and by being initially limited to three attempts removed the risk of numerous unproductive trips.

Dusky Transport

Dusky sharks were caught June 16th and 17th of 2006 via two longline sets (N37 31.628 W75 34.558 and N37 30.647 W75 34.878) with 45000 ft of 3.3 mm mono, 5 foot snoods with 4/0 circle hooks every 60 feet, soaked for 2 hours. The animal's weights ranged from 4.90kg to 7.57kg. The sharks were moved in transport tubes of two designs: 10" PVC tubes ~1m long with a top loading door and flow through intake and output tubing; and 8" PVC tubes ~0.75m long with a screw on cap section and flow through tubing. Transfer tube flow through water was collected by hull scoops and pumped into tubes by bilge pumps.

Transport Tubes



Fishery

Sandbar

Sandbar sharks are the most abundant and highly exploited shark species in the northwestern Atlantic. As a result Sminkey and Musick (1995) found population declines of 66% by 1991 on VIMS longline survey, which has been conducted since 1974. Currently recreational fishing on sandbar sharks is popular due to their easy accessibility and low gear cost requirements. Landings in this segment of the fishery have fluctuated greatly from ~11,000 animals in 2000 to ~36,000 in 2001 to ~8,000 in 2002 (NOAA 2004) but are at levels that create a significant impact. Commercial landings during this time period however, have increased from ~1,500,000 to ~1,900,000 metric tons (mt) dry weight and ~1,000mt to ~24,000mt dry weight of fins (NOAA 2004) taken legally. Shark finning has been banned in America since 1993. Sandbar sharks are now managed as part of the Large Coastal Shark division defined by the Atlantic Shark Fishery Management Plan and have shown moderate improvements in comparison to their populations prior to heavy fishery pressure.

Dusky

Dusky shark populations in the Northwest Atlantic have witnessed severe declines in the past decade. Catch rates of dusky sharks have declined from being 20% of the total catch to 1-2% in VIMS longline survey (Musick 1993). Assuming no fishing mortality and a two year reproductive cycle Cortes (1996) and Sminkey (1996) estimate population increases of 2.8% and 5.57%, respectively. These animals were designated by the National Marine Fisheries Service to be a candidate species for listing under the Endangered Species Act and have been classified by the IUCN

Red Listed of Threatened Species as vulnerable and are currently listed as at low risk but near threatened. The dusky shark is now a prohibited species and benefits from time-area closures designated off North Carolina in order to protect their nursery. The relative abundance of dusky sharks appears to be increasing (Cortes 2006).

Elasmobranch Osmoregulation

Organs Involved

The rectal gland is the major site of salt (Na^+ and Cl^-) secretion that can be hypertonic to both SW as well as the animal's own blood. However, removal of the gland by Burger (1965) cause little to no change in plasma ion levels and didn't lead to the death the spiny dogfish (Evans et al. 1982, Shuttleworth 1988, Wilson 2002). It is unknown how the animals were able to maintain their plasma ion levels in spite of the glands removal. In stenohaline FW elasmobranchs the rectal glands are non-functional, but in euryhaline animals the glands have been shown to decrease in weight and length along salinity gradients (Oguri 1964, Goldstein and Forster 1971, Gerst and Thorson 1977, Thorson et al 1978, Piermarini and Evans 1998, Pillans and Franklin 2004). It appears that as the osmoregulatory requirement to secrete sodium-chloride decreases the animals are able to reduce its physical size of the gland.

The kidney is the site of urea reabsorbtion and is what allows for urine that is hypotonic to plasma (Evans et al. 2004). Kempton (1953) showed that 70-99% of filtered urea is reabsorbed from urine in *Mustelus canis*. In response to dilute environments elasmobranchs can increase urine flow rates by 20 to 50 fold (Evans et al. 2004) in order to increase excretion of osmolytes and ions.

The large surface area of gills leads to diffusional losses of urea, however these losses are lower than those of teleosts. Fines et al. (2001) documented this by showing that the gill permeability of rainbow trout, *Oncorhynchus mykiss* exceeded the spiny dogfish by up to 60 fold. It is unknown exactly how elasmobranchs counteract these diffusional losses, possible explanations are: high cholesterol to

phospholipids ratios in the basolateral membrane vesicles (cholesterol is known to reduce urea permeability [Mourtisen and Jorgensen 1994, reviewed by Wilkie 2002 and Evans et al. 2005]); a mucus layer over the gills (Hill et al 2004); and active transport of urea from gill back into plasma (Wood et al. 1995, Part et al. 1998, Fines et al. 2001). Evans (2004) suggests that elasmobranch gill epithelium has a greater potential for active transport in FW individuals, which is consistent with their greater need for sodium-chloride uptake in FW.

Osmoregulation

Osmoregulation as defined by Hammerschlag (2006) depends on the relationship between the solute-to-solvent concentrations of the internal body fluids (extracellular and intracellular) and the outside medium that surrounds the animal. The result of a gradient existing between the animal and their environment is that water will seek to move from the area higher concentration to that of lower concentration, leading an elasmobranch moving from marine into fresh waters to risk an influx of water into their body. If the water influx isn't counteracted then the animal will experience decreased internal osmotic pressure as its osmolytes (primarily urea, TMAO, Na^+, and Cl^- [Piermarini and Evans 1998, Smith 1931, Urist 62, Thorson et. al. 1973]) become diluted as well as haemodilution as the blood and plasma also become diluted. The osmotic gain or loss of water across the gills must be balanced by increased or decreased functioning of renal, kidney, and/or gill systems. The gills are sites of net salt uptake (Piermarini et al. 2002, Wood et al. 2002), whereas the rectal gland is the major site of salt excretion and the kidney maintains the high urea and methylamine concentration by reabsorbtion and to some

lesser extent is able to reabsorb sodium and chloride (Evans et al. 2004).

Most elasmobranchs are ureotelic, with the exception of the freshwater potamotrygonid rays, which means that they synthesize and excrete urea as an end product of nitrogen metabolism (Wood et al 2002, Hazon et al. 2003). Under normal conditions elasmobranchs maintain enzyme function regardless of high urea concentrations by keeping TMAO concentrations in a 2:1 ratio where TMAO is about 50% of urea in the blood and is able to counteract the denaturing affect of urea on proteins (Hochachka and Somero 2002). On average, marine elasmobranch urea concentrations account for 30% of total plasma osmolarity (reviewed by Hammerschlag 2006) and euryhaline species in dilute medium keep low concentrations of urea and TMAO (Thorson et al. 1973, Piermarini and Evans 1998, Pillans and Franklin 2004). Sodium and chloride account for the majority of plasma osmotic pressure after urea and in marine sharks. The bull shark has been shown to have Na^+, Cl^-, and urea percent contributions of 27.1%, 27.7%, and 34.7% in SW (Pillans and Franklin 2004, reviewed by Hammerschlag 2006).

Haemodilution in these animals resulting from decreased environmental salinity can dramatically reduce hct (Goldstein and Forster 1971, Chan and Wong 1977) and result in decreased haemoglobin concentration. This anemic condition seriously impairs oxygen carrying capacity, as there is less haemoglobin available to carry oxygen and also reduces the non-bicarbonate buffering capacity of the blood and can reduce CO_2 excreting abilities (Wood et al. 1979, Cooper and Morris 2004a). Cooper and Morris (2004a) concluded that the low salinity exposure treatments promoted a lowering of mean cell haemoglobin concentration and with the loss of hct

probably account for very large reductions in CO_2 capacity and blood buffering found for the Port Jackson shark.

Literature Cited

Baum, J.K., Myers, R.A., Kehler, D.G., Worm, B., Harley, S.J., Doherty, P.A. 2003. Collapse and Conservation of Shark Populations in the Northwest Atlantic. Science. 299: 389-391.

Branstetter, S. 1990. Early life-history implications of selected carcharhinoid and lamnoid sharks of the Northwest Atlantic. NOAA Technical Report NMFS 90. 17-28

Branstetter, S., Burgess, G.H. 1996. Commercial Shark Fishery Observer Program. Characterization and comparison of the directed commercial shark fishery in the eastern Gulf of Mexico and off North Carolina through an observer program. MARFIN award NA47ff0008, 33pp+11tables+20figs.

Brill, R., Bushnell, P., Schroff, S., Seifert, R., Galvin, M. Submitted. Functional red blood cell swelling following anaerobic exercise in the sandbar shark (*Carcharhinus plumbeus,* Nardo). J. Exp. Mar. Bio. Ecol.

Burger, J.W. 1965. Roles of the rectal gland and kidneys in salt and water excretion in the spiny dogfish. Physiol. Zool. 38:191-196.

Bigelow, H.B., Schroeder, W.C. 1953. Fishes of the Gulf of Maine. Fish. Bull. 53: 577.

Carrier, J.C., Evans, D.H. 1973. Ion and water turnover in the fresh-water elasmobranch *Potamotrygon* sp. Comp. Biochem. Physiol. A. 45: 667-670.

Castro, J.I. 1983. The sharks of North American waters. Texas A&M University Press, College Station, Texas. 180 pp.

Castro, J.I. 1993. The shark nursery of Bulls Bay, South California, with a review of the shark nurseries of the southeastern coast of the United States. Environ. Biol. Fish. 38: 37-48.

Chan, D.K.O., Wong, T.M., 1977. Physiological adjustments to dilution of the external medium in the lip shark, *Hemiscyllium plagiosum* Bennet . J. Exp. Zool. 200, 97 102.

Cliff, G., Thurman, G.D. 1984. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus.* Comp. Biochem. Physiol. A.78: 167-173.

Compagno, L.J.V. 1984. FAO Species Catalogue. Vol. 4. Sharks of the World. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. 655 pp.

Compagno, L.J.V. 2001. FAO Species Catalogues. Vol. 2. Sharks of the World. An annotated and illustrated catalogue of shark species know to date. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes, and Orectolobiformes). FAO, Rome 2001. 269 pp.

Compagno, L.J.V., Dando, M., Fowler, S., 2005. Sharks of the World. Princeton University Press, Princeton, NJ. 368 pp.

Cooper, A.R., Morris, S. 1998a. Osmotic, ionic and haematological response of the Port Jackson shark *Heterodontus portusjacksoni* and the common stingaree *Trygonoptera testacea* upon exposure to diluted seawater. Mar. Biol. 132: 29-42.

Cooper, A.R., Morris, S. 1998b. The blood respiratory, haematological, acid-base and ionic status of the Port Jackson shark, *Heterodontus portusjacksoni*, during recovery from anesthesia and surgery: a comparison with sampling by direct caudal puncture. Comp. Biochem. Physiol. A. 119: 895-903.

Cooper, A.R., Morris, S. 2004a. Osmotic, sodium, carbon dioxide and acid-base state of the Port Jackson shark, *Heterodontus portusjacksoni*, in response to lowered salinity. J. Comp. Physiol. B. 174: 211-222.

Cooper, A.R., Morris, S. 2004b. Haemoglobin function and respiratory status of the Port Jackson shark, *Heterodontus portusjacksoni*. J. Comp. Physiol. B. 174: 223-236.

Cortes, E. 1998. Demographic analysis as an aid in shark stock assessment and management. Fish. Res. 39: 199-208.

Cortes, E., Brooks, E., Apostolaki, P., Brown, C.A. 2006. Stock Assessment of Dusky Shark in the U.S. Atlantic and Gulf of Mexico. Sustainable Fisheries Division Contribution.SFD-2006-014, pp. 57.

Dacie, J.V. and Lewis, S.M. 1984. Practical Haematology. Churchill Livingstone, Edinburg, New York.

DeVlaming, V.L., Sage, M. 1973. Osmoregulation in the Euryhaline Elasmobranch, *Dasyatis sabina*. Comp. Biochem. Physiol. A. 45: 31-44.

Ellis, J. Diet of the sandbar shark, *Carcharhinus plumbeus*, in Chesapeake Bay and adjacent waters. Masters. College of William and Mary, Williamsburg, VA.

Evans, D.H. 1982. Mechanisms of acid extrusion by two marine fishes: the teleosts *Opsanus beta*, and the elasmobranch, *Squalus acanthis*. J. Exp. Bio. 97: 289-299.

Evans, D.H., Peirmarini, P.M., Choe, K.P. 2004. Homeostasis: Osmoregulation, pH Regulation, and Nitrogen Excretion. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (Eds.), Biology of Sharks and Their Relatives. CRC Press, Boca Raton. pp. 247-268.

Evans, D.H., Piermarini, P.M., and Choe, K.P. 2005. The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Revs. 85: 97-177.

Fines GA, Ballantyne JS, and Wright PA. 2001. Active urea transport and an unusual basolateral membrane composition in the gills of a marine elasmobranch. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280: 16 –24.

Forster, R.P., Goldstein, L. 1976. Intracellular osmoregulatory role of amino acids and urea in marine elasmobranchs. Am. J. Physiol. 230: 925-931.

Forster, R.P., Goldstein, L., Rosen, J.K. 1972. Intrarenal control of urea reabsorbtion by renal tubules of the marine elasmobranch, *Squalus acanthis*. Comp. Biochem. Physiol. A. 42: 3-12.

Gaumet, F., Boeuf, G., Severe, A., Le Roux, A., Mayer-Gostan, N. 1995. Effects of salinity on the ionic balance and growth of juvenile turbot. J. Fish Biol. 47: 865-876.

Gelsleichter J., Musick J., Nichols S. 1999. Food habits of the smooth dogfish, *Mustelus canis*, dusky shark, *Carcharhinus obscurus*, Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, and the sand tiger, *Carcharias taurus*, from the northwest Atlantic Ocean. Environ. Biol. Fish. 54:205–217.

Gerst, J. W. and Thorson, T. B. 1977. Effects of saline acclimation on plasma electrolytes, urea excretion, and hepatic urea biosynthesis in a freshwater stingray, *Potamotrygon* sp. Garman, 1877. Comp. Biochem. Physiol. A 56: 87-93.

Goldstein, L., Forster, R.P. 1971. Osmoregulation and urea metabolism in the little skate *Raja erinacea*. Am. J. Physiol. 220: 742-746.

Grubbs, R.D. and J.A. Musick. 2007a. Spatial delineation of summer nursery areas for juvenile sandbar sharks in Chesapeake Bay, Virginia. In: C.T. McCandless, N.E. Kohler and H.L. Pratt, Jr. (Eds.). Shark nursery grounds of the Gulf of Mexico and the East Coast waters of the United States. American Fisheries Society. Bethesda, MD.

Grubbs, R.D., J.A. Musick, C. L. Conrath, and J. G. Romine. 2007b. Long-term movements, migration, and temporal delineation of summer nurseries for

juvenile sandbar sharks in the Chesapeake Bay region. In: C.T. McCandless, N.E. Kohler and H.L. Pratt, Jr. (Eds.). Shark nursery grounds of the Gulf of Mexico and the East Coast waters of the United States. American Fisheries Society. Bethesda, MD.

Hammerschlag, N. 2006. Osmoregulation in elasmobranchs: a review for fish biologists, behaviorists and ecologists. Mar. Fresh. Behav. Physiol. 39 (3): 209-228.

Harms, C., Ross, T., Segars A. 2002. Plasma biochemistry reference values of wild bonnethead sharks, *Sphyrna tiburo*. Vet. Clin. Pathol. 31: 111-115.

Hazon, N., Wells, A., Pillans, R.D., Good, J.P., Gary Anderson, W., Franklin, C.E. 2003. Urea based osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity. Comp. Biochem. Physiol. B. 136: 685-700.

Hill W.G., Mathai J.C., Gensure R.H., Zeidel J.D., Apodaca G., Saenz J.P., Kinne-Saffran E., Kinne R., Zeidel M.L. Permeabilities of teleost and elasmobranch gill apical membranes: evidence that lipid bilayers alone do not account for barrier function. Am. J. Physiol. Cell Physiol. 287: 235–242.

Hochachka, P.W., Somero, G.N. 2002. Biochemical Adaptation. Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford.

Kempton, R. T. 1953. Studies of the elasmobranch kidney. II. Reabsorption of urea by the smooth dogfish, *Mustelis canis*. 104: 45–56.

Kohler, N.E., Casey, J.G., Turner, P.A. NMFS Cooperative Shark Tagging Program, 1962-1993: An atlas of shark tag and recapture data. Mar. Fish. Rev. 60(2): 1-87.

Lawler, E.F. 1976. The biology of the sandbar shark, *Carcharhinus plumbeus*, (Nardo 1827) in the lower Chesapeake Bay and adjacent waters. Masters. College of William and Mary, Williamsburg, VA.

Martin, R.A. 2005. Conservation of freshwater and euryhaline elasmobranchs: A review. J. Mar. Biol. Assoc. UK. 85: 1049-1073.

Medved, R.J., Marshall, J.A. 1981. Feeding Behavior and Biology of Young Sandbar Sharks, *Carcharhinus plumbeus* (Pisces Carcharhinidae), in Chincoteague Bay. Fish. Bull. 79: 441-447.

Metcalf, V.J., Gemmell, N.J. 2005. Fatty acid transport in cartilaginous fish: absence of albumin and possible utilization of lipoproteins. Fish Physiol. Biochem. 31: 55-64.

Merson, R.R., Pratt, H.L. 2001. Movements and Growth of Young Sandbar Sharks, *Carcharhinus plumbeus*, in the Nursery Grounds of Delaware Bay. Environ. Biol. Fish. 61: 13 - 24.

Mouritsen O.G., Jorgensen, K. 1994. Dynamic order and disorder in lipid bilayers. Chem. Phys. Lipids 73: 3-25.

Musick J.A., Colvocoresses, J.A. 1986. Seasonal recruitment of subtropical sharks in the Chesapeake Bight, USA. Workshop on recruitment in tropical coastal demersal communities. FAO UNESCO, Campeche Mexico. 21-25 April 1986. I.O.C. Workshop Report No. 44.

Musick, J.A., Branstetter, S., Colvocoresses, J.A. 1993. Trends in shark abundance from 1974 to 1991 for the Chesapeake Bight region of the U.S. Mid-Atlantic coast. In: Branstetter, S. (Ed.), Conservation Biology of Elasmobranchs, pp. 1-18.

Musick, J.A., Burgess, G., Cailliet, G., Camhi, M., Fordham, S. 2000. Management of Sharks and Their Relatives (Elasmobranchii). Fish. 25: 9-13.

Natanson, L.J., Casey, J.G., Kohler, N.E. 1995. Age and growth estimates of the dusky shark, *Carcharhinus obscurus*, in the western North Atlantic Ocean. Fish. Bull. 93: 16-26.

Olson, K.R., Farrell, A.P. 2006. The cardiovascular system. In: Evans, D., Claiborne, J.B. (Eds.) The physiology of fishes 3rd edition. CRC Press, Boca Raton. pp. 119.

Oguri, M. 1964. Rectal glands of marine and fresh-water sharks: comparative histology. Science. 144: 1151-1152.

Part, P., Wright, P.A., Wood, C.M. 1998. Urea and water permeability in dogfish (*Squalus acanthis*) gills. Comp. Biochem. Physiol. 119: 117-123.

Piermarini, P.M., Evans, D.H. 1998. Osmoregulation of the Atlantic stingray (*Dasyatis sabina*) from the freshwater Lake Jesup of the St. Johns River, Florida. Physiol. Zool. 71: 553-560.

Piermarini, P.M., Verlander, J.W., Royaux, I.E., Evans, D.H.,2002. Pendrin immunoreactivity in the gill epithelium of a euryhaline elasmobranch. Am. J. Physiol. 283: 983-992.

Pillans, R.D., Good, J.P., Anderson, W.G., Hazon, N., Franklin, C.E. 2005. Freshwater to seawater acclimation of juvenile bull sharks (*Carcharhinus leucas*): plasma osmolytes and Na-K ATPase activity in gill, rectal gland,

kidney and intestine. J. Comp. Physiol. B. 175: 37 - 44.

Pillans, R.D., Franklin, C.E. 2004. Plasma osmolyte concentrations and rectal gland mass of bull sharks Carcharhinus leucas, captured along a salinity gradient. Comp. Biochem. Physiol. A. 138: 363-371.

Pilson, M.E.Q. 1998. An introduction to the chemistry of the sea. Prentice Hall, Upper Saddle River, NJ, 431.

Romine, J.G. 2004. Status and demographic analysis of the dusky shark, *Carcharhinus obscurus*, in the Northwest Atlantic. Masters. College of William and Mary, Williamsburg, VA.

Rechisky, E.L., Wetherbee, B.M. 2003. Short-term movements of juvenile and neonate sandbar sharks, (*Carcharhinus plumbeus*), on their nursery grounds in Delaware Bay. Environ. Biol. Fish. 68: 113 - 128.

Sampio, L.A., Bianchini, A., 2002. Salinity effects on osmoregulation and growth of the euryhaline flounder, *Paralichthys orbignyanus*. J. Exp. Mar. Biol. Ecol. 269: 187-196.

Schmidt-Nielson, B., Truniger, B., Rabinowits, L. 1972. Sodium-linked urea transport by the renal tubule of the spiny dogfish, *Squalus acanthis*. Comp. Biochem. Physiol. A. 42: 13-25.

Shuttelworth, T.J. 1988. Physiology of elasmobranch fishes. Springer-Verlag, Berlin, 324pp.

Sminkey, T.R., Musick, J.A. 1995. Age and growth of the sandbar shark, Carcharhinus plumbeus, before and after population depletion. Copeia. 871-883.

Sminkey, T.R., Musick, J.A. 1996. Demographic analysis of the sandbar shark, Carcharhinus plumbeus, in the western North Atlantic. Fish. Bull. 94: 341-347.

Smith, H.W. 1931. The absorption and excretion of water and salts by the elasmobranch fishes. I. Fresh water elasmobranchs. Am. J. Physiol. 98: 279-295.

Springer, S. 1960. Natural History of the Sandbar Shark, *Eulamia milberti*. Fish. Bull. 61: 1-36.

Sulikowski, J.A., Treberg, J.R., Howell, W.H. 2003. Fluid regulation and physiological adjustment in the winter skate, *Leucoraja ocellata*, following exposure to reduced environmental salinities. Envi. Biol. Fish. 66: 339-348.

Tam, W.L., Wong, W.P., Loong, A.M., Hiong, K.C., Chew, S.F., Ballantyne, J.S., Ip, Y.K. 2003. The osmotic response of the Asian freshwater stingray (*Himantura signifer*) to increased salinity: a comparison with marine (*Taeniura lymma*) and Amazonian freshwater (*Potamotrygon motoro*) stingrays. J. Exp. Biol. 206: 2931-2940.

Thorson, T.B., Cowan, C.M., Watson, D.E. 1973. Body Fluid Solutes of Juvenile and Adults of the Euryhaline Bull Shark *Carcharhinus leucas* from Freshwater and Saline Environments. Physiol. Zool. 46: 29-42.

Thorson, T.B., Wotton, R.M., Georgi, T.A. 1978. Rectal gland of freshwater stingrays, *Potamotrygon* spp. (Chondrichthyes: Potamotrygonidae). Biol. Bull. 154: 508-516.

Tort, L., Landri, P., Altimiras, J. 1994. Physiological and metabolic changes of sea bream Sparus aurata to short-term acclimation at low salinity. Comp. Biochem. Physiol. A. 108: 75-80.

United States Department of Commerce. 2004. Stock Assessment and Fishery Evaluation Report for Atlantic Highly Migratory Species. National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Urist, M.R. 1962. Calcium and other ions in blood and skeleton of Nicaraguan fresh water shark. Science, 137: 984-986.

Weber, R.E. 1983. TMAO-independence of oxygen affinity and its urea and ATP sensitivities in an elasmobranch haemoglobin. J. Exp. Zool. 228: 551-554.

Wilkie, M.P. 2002. Ammonia excretion and urea handling by fish gills: Present understanding and future research challenges. J. Exp. Zool. 293: 284-301.

Wilson, J.M., Morgan, J.D., Vogal, A.W., Randall, D.J. 2002. Branchial mitochondria-rich cells in the dogfish *Squalus acanthis*. Comp. Biochem. Physiol. A. 132: 78-86.

Withers, P.C., Morrison, G., Hefter, G.T., Pang, T.S. 1994. Role of urea and methylamines in buoyancy of elasmobranchs. J. Exp. Biol. 188: 175-189.

Woo, N.Y.S., Chung, K.C. 1995. Tolerance of *Pomacanthus imperator* to hypoosmotic salinities: changes in body composition and hepatic enzyme activities. J. Fish Biol. 47: 70-81.

Wood, C.M., Matsuo, A.Y., Gonzalez, R.J., Wilson, R.W., Patrick, M.L., Val., A.L. 2002. Mechanisms of ion transport in *Potamotrygon*, a stenohaline freshwater elasmobranch native to the ion-poor blackwaters of the Rio Negro. J. Exp.

Biol. 205: 3039-3054.

Wood, C.M., Part, P. Wright, P.A. 1995. Ammonia and urea metabolism in relation to gill function and acid-base balance in a marine elasmobranch, the spiny dogfish (*Squalus acanthis*). J. Exp. Biol. 198: 1545-1558.

Wood, C.M., McMahon, B.R., McDonald D.G. 1979. Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*. A comparison with other teleosts. J. Exp. Biol. 78: 167-179.

Yancey, P.H., Somero, G.N. 1979. Counteraction of urea destabilization of protein structure by methylamine osmoregulatory compounds of elasmobranch fishes. Biochem. J. 183: 317-323.

Yancey, P.H., Somero, G.N. 1980. Methylamine osmoregulatory solutes of elasmobranch fishes counteract urea inhibition of enzymes. J. Exp. Zool. 212: 205-213.

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